

Amendments to the Claims

The listing of claims will replace all prior versions and listings of claims in the application.

1. (Currently Amended) A composition comprising a conjugate comprising a peptide, protein or glycoprotein bioactive component covalently attached to at least one linear or branched polyalkylene glycol(s),

wherein ~~each~~ at least 95% of said polyalkylene glycol(s) is or are attached to said peptide, protein or glycoprotein bioactive component at a single site on said the polyalkylene glycol(s), ~~and~~

wherein a hydroxyl group is present on at least 95% of the distal polyalkylene glycol termini in said conjugate, and

wherein said conjugate in said composition exhibits reduced antigenicity compared to a conjugate comprising the same peptide, protein or glycoprotein linked at the same site or sites on the peptide, protein or glycoprotein to the same number of polyalkylene glycols of the same size and the same linear or branched structure, in which a hydroxyl group is present on less than 95% of the distal polyalkylene glycol termini in said conjugate

~~said polyalkylene glycol, if linear, has a hydroxyl group at its distal terminus or, if branched, has a hydroxyl group at every distal terminus.~~

2. (Canceled)

3. (Currently Amended) The composition ~~conjugate~~ of claim 1, wherein said linear or branched polyalkylene glycol is selected from the group consisting of a poly(ethylene glycol) and a copolymer of ethylene oxide and propylene oxide.

4. (Currently Amended) The composition ~~conjugate~~ of claim 3, wherein said linear or branched polyalkylene glycol is a poly(ethylene glycol) ("PEG").

5. (Currently Amended) The composition ~~conjugate~~ of claim 1, wherein the attachment of said polyalkylene glycol to said peptide, protein or glycoprotein ~~bioactive component~~ is carried out using a reactive derivative of at least one polyalkylene glycol selected from the group consisting of linear dihydroxyPEGs, (~~"PEG-diols"~~), hydroxyPEG-monoacetals and hydroxyPEG-monoacids.

6. (Currently Amended) The composition ~~conjugate~~ of claim 1, wherein the attachment of said polyalkylene glycol to said peptide, protein or glycoprotein ~~bioactive component~~ is carried out using a reactive derivative of hydroxyPEG selected from the group consisting of a monoaldehyde, a monoester of a monoacid, a monoamine, a monothiol, a monodisulfide, a monobromophenyl carbonate, a monochlorophenyl carbonate, a monofluorophenyl carbonate, a mononitrophenyl carbonate, a monocarbonylimidazole, a monohydrazide, a monocarbazate, a monoiodoacetamide, a monomaleimide, a monoorthopyridyl disulfide, a monooxime, a monophenyl glyoxal, a monothiazolidine-2-thione, a monothioester, a monotriazine and a monovinylsulfone.

7. (Currently Amended) The composition ~~conjugate~~ of claim 1, wherein said polyalkylene glycol has a molecular weight of from about 1,000 Daltons (1 kDa) to about 100,000 Daltons (100 kDa).

8. (Canceled)

9. (Currently Amended) The composition ~~conjugate~~ of claim 7 8, wherein said polyalkylene glycol has two branches, each with a molecular weight of from about 2 kDa to about 30 kDa.

10. (Currently Amended) The composition ~~conjugate~~ of claim 9, wherein said polyalkylene glycol has two branches, each with a molecular weight of from about 5 kDa to about 20 kDa.

11. (Currently Amended) The composition ~~conjugate~~ of claim 7 8, wherein said polyalkylene glycol has a molecular weight of from about 10 kDa to about 20 kDa.

12. (Currently Amended) The composition ~~conjugate~~ of claim 11, wherein said polyalkylene glycol has a molecular weight of about 12 kDa.

13. (Currently Amended) The composition ~~conjugate~~ of claim 7 8, wherein said polyalkylene glycol has a molecular weight of from about 18 kDa to about 60 kDa.

14. (Currently Amended) The composition ~~conjugate~~ of claim 13, wherein said polyalkylene glycol has a molecular weight of from about 18 kDa to about 22 kDa.

15. (Currently Amended) The composition ~~conjugate~~ of claim 14 wherein said polyalkylene glycol has a molecular weight of about 20 kDa.

16. (Currently Amended) The composition ~~conjugate~~ of claim 13, wherein said polyalkylene glycol has a molecular weight of about 27 kDa to about 33 kDa.

17. (Currently Amended) The composition ~~conjugate~~ of claim 1, wherein said ~~conjugate comprises~~ peptide, protein or glycoprotein is attached to from ~~about~~ one to about 100 ~~strands~~ molecules of said polyalkylene glycol.

18. (Currently Amended) The composition ~~conjugate~~ of claim 17, wherein said ~~conjugate comprises~~ peptide, protein or glycoprotein is attached to from ~~about~~ one to about five ~~strands~~ molecules of said polyalkylene glycol.

19. (Currently Amended) The composition ~~conjugate~~ of claim 18, wherein said ~~conjugate comprises~~ peptide, protein or glycoprotein is attached to ~~about~~ one or ~~about~~ two ~~strands~~ molecules of said polyalkylene glycol.

20. (Currently Amended) The composition ~~conjugate~~ of claim 17, wherein said ~~conjugate comprises~~ peptide, protein or glycoprotein is attached to about five to about 100 ~~strands~~ molecules of said polyalkylene glycol.

21. (Currently Amended) The composition ~~conjugate~~ of claim 1, wherein said polyalkylene glycol is selected from the group consisting of a monohydroxyPEG-acid and a dihydroxyPEG-acid.

22. (Canceled)

23. (Currently Amended) The composition ~~conjugate~~ of claim 5, wherein said polyalkylene glycol is a reactive derivative of said linear dihydroxyPEG.

24. (Currently Amended) The composition ~~conjugate~~ of claim 5, wherein said polyalkylene glycol is a reactive derivative of said hydroxyPEG-monoacid.

25. - 34. (Canceled)

35. (Currently Amended) The composition ~~conjugate~~ of claim 1 ~~26~~, wherein said peptide, protein or glycoprotein is an allergen.

36. (Canceled)

37. (Canceled)

38. (Currently Amended) ~~A pharmaceutical~~ The composition of claim 1, wherein said composition is a pharmaceutical composition comprising ~~the conjugate of claim 1 and~~ a pharmaceutically acceptable excipient or carrier.

39. - 58. (Canceled)

59. (Currently Amended) ~~A conjugate comprising a bioactive component~~ The composition of claim 1, wherein said peptide, protein or glycoprotein is covalently attached to at least one linear or branched polyalkylene glycol that had been converted to a monofunctionally activated polyalkylene glycol ~~activated at only one terminus ("a monofunctionally activated polyalkylene glycol")~~ produced by a method comprising:

(a) obtaining a polyalkylene glycol that has a hydroxyl group at every terminus;

(b) ~~optionally,~~ prior to the conversion of the polyalkylene glycol of (a) to a monofunctionally activated polyalkylene glycol, protecting all except one of the hydroxyl groups in said polyalkylene glycol by the addition of one or more removable

blocking groups, ~~such as *t*-butoxyl group(s), aryloxyl group(s) or triphenylmethyl group(s) ("trityl group(s)");~~

(c) producing a monofunctionally activated derivative of said polyalkylene glycol by reacting said polyalkylene glycol with a derivatizing compound or compounds under conditions such that said polyalkylene glycol is derivatized with a single derivatizing group at a hydroxyl group that does not contain said removable blocking group or groups;

~~(d) if a blocking group was added to protect the hydroxyl group(s) in (b);~~

(d) purifying said monofunctionally activated derivative;

(e) removing said blocking group without removing the derivatizing ~~activating~~ group attached in (c), to produce a monofunctionally activated polyalkylene glycol wherein the distal terminus or distal termini are hydroxyl group(s); and

(e) (f) contacting said monofunctionally activated polyalkylene glycol with said peptide, protein or glycoprotein ~~a bioactive component~~, under conditions that favor the covalent binding of said monofunctionally activated polyalkylene glycol to said peptide, protein or glycoprotein ~~bioactive component~~, or

~~(f) alternatively, performing said contacting (e) prior to performing said removing (d).~~

60. (Canceled)

61. (Currently Amended) The composition ~~conjugate~~ of claim 59, wherein said polyalkylene glycol is selected from the group consisting of a poly(ethylene glycol) and a copolymer of ethylene oxide and propylene oxide.

62. (Currently Amended) The composition ~~conjugate~~ of claim 59, wherein the polyalkylene glycol component is selected from the group consisting of a linear poly(ethylene glycol) and a branched poly(ethylene glycol).

63. (Currently Amended) The composition ~~conjugate~~ of claim 59, wherein each said polyalkylene glycol has a molecular weight of from about 1 kDa to about 100 kDa.

64. (Canceled)

65. (Currently Amended) The composition ~~conjugate~~ of claim ~~64~~ 63, wherein said polyalkylene glycol has two branches, each with a molecular weight of from about 2 kDa to about 30 kDa.

66. (Canceled)

67. (Currently Amended) The composition ~~conjugate~~ of claim ~~64~~ 63, wherein said polyalkylene glycol has a molecular weight of from about 10 kDa to about 20 kDa.

68. – 72. (Canceled)

73. (Currently Amended) The composition ~~conjugate~~ of claim 59, wherein said ~~conjugate comprises~~ peptide, protein or glycoprotein is attached to from one to about 100 ~~strands~~ molecules of said polyalkylene glycol.

74. (Currently Amended) The composition ~~conjugate~~ of claim 73, wherein said ~~conjugate comprises about~~ peptide, protein or glycoprotein is attached to from one to about five ~~strands~~ molecules of said polyalkylene glycol.

75. (Currently Amended) The composition ~~conjugate~~ of claim 74, wherein said ~~conjugate comprises about~~ peptide, protein or glycoprotein is attached to one or about two ~~strands~~ molecules of said polyalkylene glycol.

76. (Currently Amended) The composition ~~conjugate~~ of claim 73, wherein said ~~conjugate comprises~~ peptide, protein or glycoprotein is attached to about five to about 100 ~~strands~~ molecules of said polyalkylene glycol.

77. (Currently Amended) The composition ~~conjugate~~ of claim 59, wherein said monofunctionally activated polyalkylene glycol ~~used in the synthesis of said conjugate~~ is selected from the group consisting of a hydroxyPEG-monoaldehyde and a reactive ester of a hydroxyPEG-monoacid.

78. (Canceled)

79. (Currently Amended) The composition ~~conjugate~~ of claim 59, wherein said monofunctionally activated polyalkylene glycol ~~used in its synthesis~~ is derived from a linear dihydroxyPEG.

80. - 89. (Canceled)

90. (Currently Amended) The composition ~~conjugate~~ of claim 59 ~~81~~, wherein said peptide, protein or glycoprotein is an allergen.

91. (Canceled)

92. (Canceled)

93. (Canceled)

94. (Currently Amended) A kit comprising more than one container, at least one of said containers comprising the composition ~~conjugate~~ of claim 1.

95. - 108. (Canceled)

109. (Currently Amended) The composition ~~conjugate~~ of claim 21, wherein said dihydroxyPEG-acid is dihydroxyPEG-lysine.

110. (Currently Amended) The composition ~~conjugate~~ of claim 1 ~~26~~, wherein said peptide, ~~or~~ protein or glycoprotein is selected from the group consisting of an enzyme, a serum protein, a serum glycoprotein, a blood cell protein, a pigmentary protein, hemoglobin, a viral protein, a peptide hormone, a protein hormone, a glycoprotein hormone, a hypothalamic releasing factor, a cytokine and a growth factor.

111. (Currently Amended) The composition ~~conjugate~~ of claim 110, wherein said serum protein is selected from the group consisting of an albumin, an immunoglobulin and a blood clotting factor.

112. (Currently Amended) The composition ~~conjugate~~ of claim 110, wherein said peptide hormone or protein hormone or glycoprotein hormone is selected from the group consisting of an antidiuretic hormone, chorionic gonadotropin, luteinizing hormone, follicle-stimulating hormone, insulin, prolactin, a somatomedin, growth hormone, thyroid-stimulating hormone and a placental lactogen.

113. (Currently Amended) The composition ~~conjugate~~ of claim 110, wherein said growth factor is selected from the group consisting of a colony-stimulating factor, an epidermal growth factor, erythropoietin, a fibroblast growth factor, an insulin-like

growth factor, a transforming growth factor, a platelet-derived growth factor, a nerve growth factor, a hepatocyte growth factor, a neurotrophic factor, a ciliary neurotrophic factor, a brain-derived neurotrophic factor, a glial-derived neurotrophic factor and a bone morphogenic peptide.

114. (Currently Amended) The composition ~~conjugate~~ of claim 110, wherein said cytokine is selected from the group consisting of a lymphokine, an interleukin, an interferon, a tumor necrosis factor, a leukemia inhibitory factor and thrombopoietin.

115. (Currently Amended) The composition ~~conjugate~~ of claim 110, wherein said enzyme is selected from the group consisting of a carbohydrate-specific enzyme, a proteolytic enzyme, an oxidoreductase, a transferase, a hydrolase, a lyase, an isomerase and a ligase.

116. (Currently Amended) The composition ~~conjugate~~ of claim 115, wherein said oxidoreductase is a uricase.

117. (Currently Amended) The composition ~~conjugate~~ of claim 115, wherein said proteolytic enzyme is a plasminogen activator.

118. (Currently Amended) The composition ~~conjugate~~ of claim 59 ~~84~~, wherein said peptide, protein or glycoprotein is selected from the group consisting of an enzyme, a serum protein, a serum glycoprotein, a blood cell protein, a pigmentary protein, hemoglobin, a viral protein, a peptide hormone, a protein hormone, a glycoprotein hormone, a hypothalamic releasing factor, a cytokine and a growth factor.

119. (Currently Amended) The composition ~~conjugate~~ of claim 118, wherein said serum protein is selected from the group consisting of an albumin, an immunoglobulin and a blood-clotting factor.

120. (Currently Amended) The composition ~~conjugate~~ of claim 118, wherein said peptide hormone or protein hormone or glycoprotein hormone is selected from the group consisting of an antidiuretic hormone, chorionic gonadotropin, luteinizing hormone, follicle-stimulating hormone, insulin, prolactin, a somatomedin, growth hormone, thyroid-stimulating hormone and a placental lactogen.

121. (Currently Amended) The composition ~~conjugate~~ of claim 118, wherein said growth factor is selected from the group consisting of a colony-stimulating factor, an epidermal growth factor, erythropoietin, a fibroblast growth factor, an insulin-like growth factor, a transforming growth factor, a platelet-derived growth factor, a nerve growth factor, a hepatocyte growth factor, a neurotrophic factor, a ciliary neurotrophic factor, a brain-derived neurotrophic factor, a glial-derived neurotrophic factor and a bone morphogenic peptide.

122. (Currently Amended) The composition ~~conjugate~~ of claim 118, wherein said cytokine is selected from the group consisting of a lymphokine, an interleukin, an interferon, a tumor necrosis factor, a leukemia inhibitory factor and thrombopoietin.

123. (Currently Amended) The composition ~~conjugate~~ of claim 118, wherein said enzyme is selected from the group consisting of a carbohydrate-specific enzyme, a proteolytic enzyme, an oxidoreductase, a transferase, a hydrolase, a lyase, an isomerase and a ligase.

124. (Currently Amended) The composition ~~conjugate~~ of claim 123, wherein said oxidoreductase is a uricase.

125. (Currently Amended) The composition ~~conjugate~~ of claim 123, wherein said proteolytic enzyme is a plasminogen activator.

126. (Currently Amended) The composition ~~conjugate~~ of claim 110, wherein said growth factor is a colony-stimulating factor.

127. (Currently Amended) The composition ~~conjugate~~ of claim 126, wherein said colony-stimulating factor is a granulocyte-macrophage colony-stimulating factor (GM-CSF).

128. (Currently Amended) The composition ~~conjugate~~ of claim 127, wherein said GM-CSF is covalently attached to one linear or branched polyalkylene glycol molecule ~~that, if linear, has a hydroxyl group at its distal terminus, or, if branched, has a hydroxyl group at every distal terminus.~~

129. (Currently Amended) The composition ~~conjugate~~ of claim 127, wherein said GM-CSF is covalently attached to two linear or branched polyalkylene glycol molecules ~~each of which, if linear, has a hydroxyl group at its distal terminus, or, if branched, has a hydroxyl group at every distal terminus.~~

130. (Currently Amended) The composition ~~conjugate~~ of claim 118, wherein said growth factor is a colony-stimulating factor.

131. (Currently Amended) The composition ~~conjugate~~ of claim 130, wherein said colony-stimulating factor is a GM-CSF.

132. (Canceled)

133. (Canceled)

134. (Currently Amended) The composition ~~conjugate~~ of claim 113, wherein said growth factor is erythropoietin.

135. (Canceled)

136. (Canceled)

137. (Currently Amended) The composition ~~conjugate~~ of claim 121, wherein said growth factor is erythropoietin.

138. (Canceled)

139. (Canceled)

140. (New) The composition of claim 18, wherein said peptide, protein or glycoprotein is attached to from one to three molecules of said polyalkylene glycol.

141. (New) The composition of claim 59, wherein said peptide, protein or glycoprotein is attached to from one to three molecules of said polyalkylene glycol.

142. (New) The composition of claim 1, wherein a hydroxyl group is present on at least 98% of the distal polyalkylene glycol termini in said conjugate.

143. (New) The composition of claim 1, wherein a hydroxyl group is present on at least 99% of the distal polyalkylene glycol termini in said conjugate.

144. (New) The composition of claim 59, wherein said one or more removable blocking groups is or are selected from the group consisting of *t*-butoxyl groups, aryloxy groups, and triphenylmethyl groups.

145. (New) The composition of claim 59, wherein said contacting (f) is performed prior to said removing (e).

146. (New) The composition of claim 1, wherein said peptide, protein or glycoprotein is covalently attached to at least one linear or branched polyalkylene glycol that had been activated at only one terminus produced by a method comprising:

(a) obtaining a polyalkylene glycol that has a hydroxyl group at every terminus;

(b) producing an activated derivative of said polyalkylene glycol by reacting said polyalkylene glycol with a derivatizing compound or compounds under conditions such that a derivatized polyalkylene glycol is formed;

(c) purifying said activated derivative of said polyalkylene glycol; and

(d) contacting said activated derivative of said polyalkylene glycol with said peptide, protein or glycoprotein under conditions that favor the covalent binding of said activated derivative of said polyalkylene glycol to said peptide, protein or glycoprotein.

147. (New) The composition of claim 146, wherein said polyalkylene glycol is selected from the group consisting of a poly(ethylene glycol) and a copolymer of ethylene oxide and propylene oxide.

148. (New) The composition of claim 146, wherein the polyalkylene glycol component is selected from the group consisting of a linear poly(ethylene glycol) and a branched poly(ethylene glycol).

149. (New) The composition of claim 146, wherein each said polyalkylene glycol has a molecular weight of from about 1 kDa to about 100 kDa.

150. (New) The composition of claim 149, wherein said polyalkylene glycol has two branches, each with a molecular weight of from about 2 kDa to about 30 kDa.

151. (New) The composition of claim 149, wherein said polyalkylene glycol has a molecular weight of from about 10 kDa to about 20 kDa.

152. (New) The composition of claim 146, wherein said peptide, protein or glycoprotein is attached to from one to about 100 molecules of said polyalkylene glycol.

153. (New) The composition of claim 152, wherein said peptide, protein or glycoprotein is attached to from one to about five molecules of said polyalkylene glycol.

154. (New) The composition of claim 152, wherein said peptide, protein or glycoprotein is attached to one or two molecules of said polyalkylene glycol.

155. (New) The composition of claim 152, wherein said peptide, protein or glycoprotein is attached to about five to about 100 molecules of said polyalkylene glycol.

156. (New) The composition of claim 146, wherein said activated derivative of said polyalkylene glycol is selected from the group consisting of a hydroxyPEG-monoaldehyde and a reactive ester of a hydroxyPEG-monoacid.

157. (New) The composition of claim 146, wherein said polyalkylene glycol is derived from a linear dihydroxyPEG.

158. (New) The composition of claim 146, wherein said peptide, protein or glycoprotein is an allergen.

159. (New) A pharmaceutical composition comprising the composition of claim 146 and a pharmaceutically acceptable excipient or carrier.

160. (New) The composition of claim 159, wherein said peptide, protein or glycoprotein is selected from the group consisting of an enzyme, a serum protein, a serum glycoprotein, a blood cell protein, a pigmentary protein, hemoglobin, a viral protein, a peptide hormone, a protein hormone, a glycoprotein hormone, a hypothalamic releasing factor, a cytokine and a growth factor.

161. (New) The composition of claim 160, wherein said serum protein is selected from the group consisting of an albumin, an immunoglobulin and a blood-clotting factor.

162. (New) The composition of claim 160, wherein said peptide hormone or protein hormone or glycoprotein hormone is selected from the group consisting of an antidiuretic hormone, chorionic gonadotropin, luteinizing hormone, follicle-stimulating hormone, insulin, prolactin, a somatomedin, growth hormone, thyroid-stimulating hormone and a placental lactogen.

163. (New) The composition of claim 160, wherein said growth factor is selected from the group consisting of a colony-stimulating factor, an epidermal growth factor, erythropoietin, a fibroblast growth factor, an insulin-like growth factor, a transforming

growth factor, a platelet-derived growth factor, a nerve growth factor, a hepatocyte growth factor, a neurotrophic factor, a ciliary neurotrophic factor, a brain-derived neurotrophic factor, a glial-derived neurotrophic factor and a bone morphogenic peptide.

164. (New) The composition of claim 160, wherein said cytokine is selected from the group consisting of a lymphokine, an interleukin, an interferon, a tumor necrosis factor, a leukemia inhibitory factor and thrombopoietin.

165. (New) The composition of claim 160, wherein said enzyme is selected from the group consisting of a carbohydrate-specific enzyme, a proteolytic enzyme, an oxidoreductase, a transferase, a hydrolase, a lyase, an isomerase and a ligase.

166. (New) The composition of claim 165, wherein said oxidoreductase is a uricase.

167. (New) The composition of claim 165, wherein said proteolytic enzyme is a plasminogen activator.

168. (New) The composition of claim 160, wherein said growth factor is a colony-stimulating factor.

169. (New) The composition of claim 168, wherein said colony-stimulating factor is a GM-CSF.

170. (New) The composition of claim 160, wherein said growth factor is erythropoietin.

171. (New) The composition of claim 146, wherein said peptide, protein or glycoprotein is attached to from one to three molecules of said polyalkylene glycol.